ISSN: 1538-4101 (Print) 1551-4005 (Online) Journal homepage: https://www.tandfonline.com/loi/kccy20

Regulation of Myc by miR-34c: A mechanism to prevent genomic instability?

Ian Cannell & Martin Bushell

To cite this article: Ian Cannell & Martin Bushell (2010) Regulation of Myc by miR-34c: A mechanism to prevent genomic instability?, Cell Cycle, 9:14, 2798-2802, DOI: 10.4161/cc.9.14.12182

To link to this article: https://doi.org/10.4161/cc.9.14.12182

Cell Cycle 9:14, 2726-2730; July 15, 2010; © 2010 Landes Bioscience

Regulation of Myc by miR-34c

A mechanism to prevent genomic instability?

Ian G. Cannell[1] and Martin Bushell[2,*]

[1]David H. Koch Institute for Integrative Cancer Research; Massachusetts Institute of Technology; Cambridge, MA USA; [2]MRC Toxicology Unit; University of Leicester; Leicester UK

Over the past 8 years several lines of compelling evidence have indicated that microRNAs are critical downstream effectors of classic oncogene/tumor suppressor networks. The archetypal examples of oncogene and tumor suppressor microRNAs are the miR-17-92 (oncomir1) polycistron and miR-34 respectively. Whilst the involvement of these two opposing families of microRNAs in oncogenesis has been known for some time, the mRNA targets through which they exert their phenotypes are only just beginning to be uncovered. Moreover, several recent reports have demonstrated that the relevant physiological targets of certain individual microRNAs are actually fairly limited, with repression of just one or two major targets sufficient to explain the observed phenotype. In this review we will discuss the emerging role of microRNAs in tumorigenesis with a specific focus on miR-34c-dependent regulation of Myc.

Key words: microRNA, cell cycle, DNA damage, P38

Submitted: 04/26/10

Accepted: 04/27/10

Previously published online: www.landesbioscience.com/journals/cc/article/12182

DOI: 10.4161/cc.9.14.12182

*Correspondence to: Martin Bushell; Email: mb446@leicester.ac.uk

Do MicroRNAs Act through the Many or the Few?

MicroRNAs are non-coding RNA molecules 18–24 nucleotides in length that regulate gene expression. They generally function by binding with imperfect Watson-Crick base pairing to target sequences within the 3' UTR of protein coding mRNAs leading to their translational repression.[1] There are over 700 identified microRNAs in the human genome which are, themselves, subject to regulation at the transcriptional and post-transcriptional levels. MicroRNAs are generally transcribed by RNA Polymerase II (Pol II) and are regulated by transcription factors, transcriptional co-activators and chromatin modifications much like any protein coding Pol II transcript. Once produced the primary microRNA (pri-miR) transcript is spliced and/or processed by the enzyme Drosha to form a precursor microRNA (pre-miR) which is exported from the nucleus and further processed by the RNAse III enzyme Dicer to form the mature microRNA duplex.[2] One strand of this duplex is loaded into one of the Argonaute protein family members (AGO1-4 in mammals) which recruits other factors such as GW182 (TNRC6A-C) to form a microRNA-induced silencing complex (miRISC). MiRISC is recruited to the 3' UTR[1] of mRNA targets by virtue of microRNA-mRNA base pairing, thus eliciting translational repression and/or mRNA destabilisation. The magnitude of translational repression appears to differ between microRNA-mRNA pairs for reasons that remain unknown, although there is evidence that sequence specific RNA binding proteins have a role to play.[3] Indeed, the precise mechanism by which microRNAs repress the translation of their target mRNAs is a matter of debate with many factors being implicated in this process.[1]

Each individual microRNA in the genome is predicted to regulate, on average, upwards of 200 protein coding mRNAs by binding to target sites within the 3' UTR. However, identifying the mRNAs that are true physiological targets of a given microRNA is a major challenge within the field. It has been proposed that microRNAs exert their function by acting as rheostats to fine-tune gene expression

through modestly (10–20%) repressing a large number of mRNAs and this is supported by large scale proteomic studies.[4,5] However, there is increasing evidence that the observed phenotype of certain microRNAs that act as master regulators of cell fate can be explained by the substantial repression (50–70%) of a fairly limited (2–3) repertoire of important mRNA targets. For example, miR-31 inhibits tumor metastasis, a phenotype that can be explained by repression of just three targets; RhoA, integrin-alpha5 and radixin.[6,7] Similarly, the pro-metastatic microRNA miR-10b appears to function primarily by repressing HOXD10, since the phenotype of miR-10b inhibition is reversed when HOXD10 is depleted by RNAi.[8] Using "knock-in" technology two groups simultaneously demonstrated that expression of an activation induced cytidine-deaminase (AID) mRNA with a mutant miR-155 target-site in its 3' UTR partially phenocopies deletion of miR-155 in vivo.[9,10] Taken together these observations suggest that certain microRNAs act on a subset of critical mRNA targets which are necessary and sufficient to explain the majority of their effects, although this does not rule out the possibility that the modest repression of a more substantial number of mRNAs contributes to both the magnitude and robustness of the phenotype. Our recent data suggest that Myc is a major physiological target of miR-34c in response to DNA damage-inducing chemotherapy,[11] providing mechanistic insight into the anti-proliferative effects of this tumor suppressor microRNA.[12,13]

Myc is a Major Target of miR-34c in Response to DNA Damage

The proto-oncogene c-Myc (hereafter referred to as Myc) is a master regulator of cell proliferation and transformation through both transcriptional and non-transcriptional means and is frequently deregulated in human malignancies. As a transcription factor Myc binds to E-box elements (with its partner Max) and activates the transcription of genes whose products function to drive cell cycle progression and cell growth. Myc also negatively regulates the transcription of genes which function to arrest the cell cycle, primarily through the Myc-associated zinc finger protein, Miz1.[14,15] In addition to regulating protein coding mRNAs, Myc transcriptionally regulates a number of non-coding RNA transcripts including ribosomal RNAs, tRNAs and microRNAs.[16] Myc has been a major focus of microRNA research for many years. It globally represses microRNA transcription,[17] inhibits the biogenesis of the tumor suppressor microRNA let-7 through Lin-28,[18] and specifically activates certain oncogenic microRNAs such as miR-17-92.[19,20] MiR-17-92 is a polycistronic microRNA cluster consisting of miR-17, 18a, 19a, 20a, 19b and 92a that is transcriptionally activated by Myc,[19] co-operates with Myc to accelerate B-cell lymphomagenesis[20] and augments tumor angiogenesis.[21] Until recently it was unknown whether the oncogenicity of miR-17-92 was due to the concerted action of all its components or whether it could be attributed to a single microRNA. Two recent reports demonstrated that miR-19 is the principal oncogenic component of this cluster in vivo and suggest that the tumor suppressor PTEN is a major target of miR-19.[22-24] Myc also has non-transcriptional functions. It directly interacts with components of the replication machinery to positively regulate DNA synthesis. Overexpression of Myc in this context causes inappropriate replication origin firing leading to replication stress and genomic instability evidenced by the activation of a DNA damage response.[25] This is particularly important since genomic instability is a hallmark of cancer[26] and is evident in Myc-driven tumors in vivo.[27] Clearly these pleiotropic functions mean Myc must be tightly controlled in order to prevent tumorgenesis, although Myc alone cannot transform cells and requires at least one co-operating oncogene.[28]

The miR-34 family comprising miR-34a, 34b and 34c, were first identified as direct transcriptional targets of p53.[12,29-31] MiR-34a is the most well characterised family member and is transcribed from a separate locus to miR-34b/c which are produced as a single primary transcript.[13] All three of these microRNAs have potent anti-proliferative affects with miR-34a additionally promoting apoptosis.[13] There are numerous reports of alterations in miR-34 expression in cancer highlighting the importance of this microRNA family in oncogenesis. For example, miR-34a is deleted in ~30% of neuroblastomas and the miR-34b/c promoter region is frequently epigentically silenced in metastatic cancer cell lines. (For further examples of miR-34 alterations in cancer see **Table 1**). The different members of the miR-34 family target several cell cycle regulators although which of these mRNAs is/are responsible for their anti-proliferative affects and whether the three members have distinct or overlapping targets is only starting to be elucidated. Furthermore, the precise role of miR-34 in the DNA damage response is yet to be fully defined.

We have recently shown that Myc is negatively regulated by miR-34c in response to DNA damage and that this is a crucial event in limiting Myc-induced DNA synthesis. Inhibition of miR-34c following DNA damage prevents S-phase arrest and leads to increased DNA synthesis, DNA damage and checkpoint activation. Remarkably, despite the number of predicted (and known) mRNA targets of miR-34c all these phenotypes of miR-34c inhibition are reversed when miR-34c is inhibited in combination with depletion of Myc by RNAi.[11] These data suggest that following DNA damage Myc is a major target of miR-34c and have important implications for our understanding of Myc biology. Continued replication under stress conditions may be one mechanism by which Myc promotes genomic instability and achieves its oncogenic potential.[32] Our data argue that miR-34c lies at the centre of a pathway that acts to remove Myc in order to prevent this oncogenic function (**Fig. 2**). Whilst Myc is clearly an important target of miR-34c we cannot rule out the possibility that other miR-34c targets are important in different cellular contexts. However, several other miR-34c targets, both experimentally validated and predicted, are also transcriptional targets of Myc (www.myc-cancer-gene.org) so these targets may be controlled by miR-34c both directly (by translational repression via their 3' UTR) and indirectly (by a reduction in transcription due a decrease in Myc levels). One target of miR-34c that

Table 1. The miR-34 family in human cancer

Cancer type	Family member	Up/downregulation (compared to normal tissue)	% of primary tumors (if known)	Biological function	Ref
Colorectal cancer	miR-34b, miR-34c	Down, Hyper-methylated	90%	Inhibition of cell growth	51
High grade serous ovarian carcinomas	miR-34c	Down, Unkown mechanism	Unknown	Low Expression correlates with poor survival	52
Osteosarcoma	miR-34a, miR-34b, miR-34c	Down, Small degree of loss of heterzygosity, small degree of methylation	~20%	Increase in G_1, promotes apoptosis	53
Colon, melanoma, head and neck, lung, breast	miR-34b, miR-34c	Down, Hypermethylated	60% methylated in metastatic primary tumors versus 40% methylated in non-metastatic primary tumors	Inhibition of cell migration	54
Prostate	miR-34a	Down, Hypermethylation	79.1%	Increase in G_1, promotes apoptosis	55
Neuroblastoma	miR-34a	Down, Deletion of 1p36.23	~30%	Promotes apoptosis	56–58
Epithelial Ovarian cancer	miR-34a, miR-34b, miR-34c	Down, Hypermethylation and copy number changes	miR-34a 100% and miR-34b/c 72% in cases with p53 mutation. miR-34b/c reduced in advanced tumors	Decrease proliferation and invasion in cells with p53 mutation	59
Breast, lung	miR-34b, miR-34c	Deletion of 11q23-q24	~48%	Unknown	60

cannot be ignored is Bcl-2, whilst neither Myc nor Bcl-2 on their own can convert cells to a tumorigenic state, together these potent oncogenes can co-operate to transform cells.[28,33] The question of whether genetic loss of miR-34c alone leads to tumorgenesis in vivo, however, awaits the miR-34c "knock-out" mouse.

Pathways Upstream of miR-34c-Myc

Double-strand breaks to DNA caused by ionizing radiation or chemotherapeutic drugs initiate a DNA damage response.[34] The DDR links the recognition of DNA damage sites to cell cycle arrest (to allow DNA repair) or apoptosis (to eliminate the damaged cell) through a series of kinase signalling networks.[35] Double-strand breaks are recognized by the MRN complex (consisting of Mre11, Rad50 and Nbs1) which, in conjunction with phosphorylated H2AX, amplifies the DNA damage signal. This in turn leads to local activation of the phosphotidyl inositol-3 kinase like (PI3K) family members ATM/ATR, which transduce the signal to the downstream checkpoint kinases CHK1, CHK2 and p38 MAPK/MAPKAPK2. These checkpoint kinases, through phosphorylation of their substrates, initiate cell cycle arrest thus allowing DNA repair.[34] Cancer cells frequently bypass one or more of these checkpoint pathways rendering such cells reliant upon the remaining checkpoints to maintain DNA damage below a threshold which induces cell death. In particular MK2 appears to be essential for S-phase and G_2/M arrest in cells that are devoid of p53, and its inhibition specifically sensitises p53-deficient cells to DNA damage-induced cell death.[36] Therefore it would appear that checkpoint kinases and perhaps their downstream effectors may be attractive therapeutic candidates in specific cellular contexts.[37] The miR-34 family were first identified as direct transcriptional targets of p53, although induction of miR-34b/c in response to DNA damage[11] and miR-34a in response to B-Raf induced senescence[38] still occur in the absence of p53. We found that in the absence of p53 the p38 MAPK pathway, through MK2, controls miR-34b/c induction in response to DNA damage[11] raising the possibility that miR-34c may function as part of the MK2-mediated S-phase checkpoint. Indeed miR-34 induction in response to DNA damage in *C. elegans* is identical in wild-type and cep1/p53 mutant strains[39] suggesting that another mechanism, perhaps involving p38 MAPK/MK2, evolved prior to the p53-dependent mechanism. Since p38 MAPK is a general stress-response kinase activated by a diverse range of stimuli,[40] it is possible that the p38 MAPK/MK2 acting through miR-34c may control Myc-dependent DNA synthesis in response to a variety of different stresses. Indeed induction of miR-34c and repression of Myc occur in response to sodium arsenite treatment (**Fig. 1**), a general p38 MAPK activator.[41] We propose that the p38 MAPK/MK2/miR-34c pathway functions to prevent Myc-dependent DNA replication under stress. Loss of this regulatory mechanism may facilitate replication under stress and promote genomic instability, a potentially oncogenic function of Myc[32,42,43] (**Fig. 2**).

Biological Significance of miR-34c-Mediated Repression of Myc

Members of the miR-34 family are known to induce a senescent-like growth arrest when overexpressed in cells, with miR-34c being the most potent.[12] We provided mechanistic insight into this striking effect by showing that miR-34c inhibition of Myc in response to DNA damage prevents S-phase progression. Thus miR-34c through repression of Myc

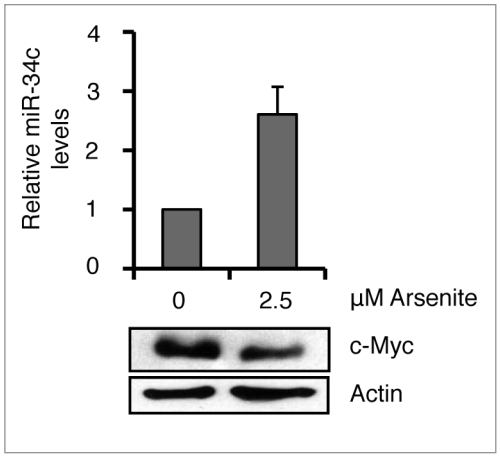

Figure 1. MiR-34c is upregulated and Myc is repressed in response to sodium arsenite. HEK293 cells were treated with 2.5 μM sodium arsenite for 8 hrs and levels of miR-34c and Myc determined by qPCR and western blot respectively.

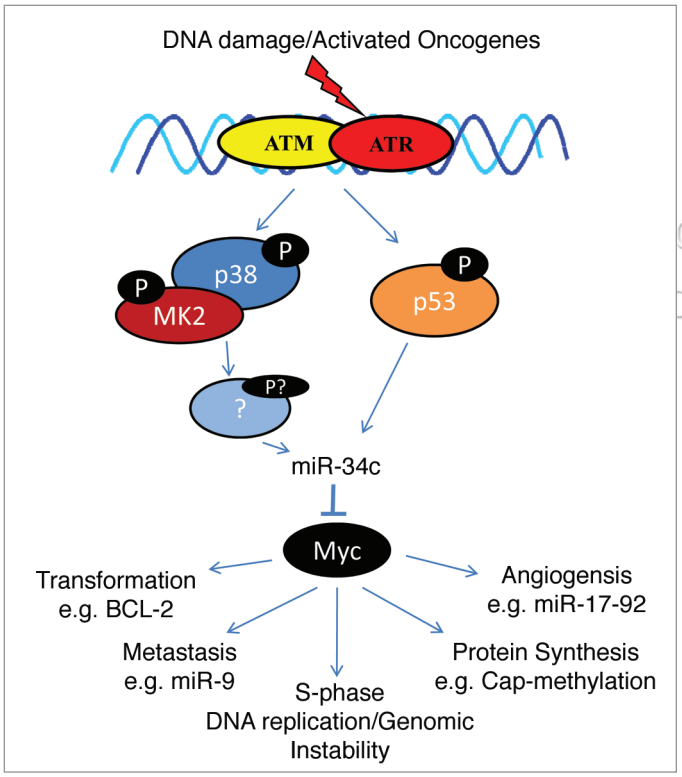

Figure 2. The miR-34c-Myc pathway and downstream biological functions.

prevents continued DNA synthesis and proliferation in the presence of damaged DNA.[11] These data have important implications, since activated oncogenes can promote genomic instability and engage a DNA damage response (DDR) leading to oncogene-induced senescence, a permanent growth arrest and innate tumor suppressor mechanism.[44] Oncogenic Ras, for example, requires continued high levels of Myc in order to bypass senescence and transform cells.[45] Under conditions in which miR-34c induction is intact activation of the DDR by Ras would repress Myc and induce senescence, however loss of this regulatory mechanism could lead to a bypass of this growth arrest, continued proliferation and genomic instability. This idea is particularly pertinent in the context of pancreatic ductal adenocarcinoma (PDAC). In a mouse model of PDAC an initial activating mutation in KRAS is followed by senescence, however a second mutation in p53 (creating mutant p53(R175H)) bypasses this growth arrest and such cells progress to PDAC.[46] Mutation of p53 (R175H) leads to the expression of a stable protein with dominant-negative functions.[47] Since miR-34c is a p53 target gene it is tempting to speculate that mutant p53 may inhibit miR-34c induction, leading to upregulation of Myc and bypass of senescence. Interestingly, unlike complete loss of p53, mutant p53 (R175H) possess gain-of-function attributes including promotion of invasion and metastases.[48] Whilst hyper-methylation of the miR-34b/c promoter is well documented in metastatic cancer cells (see **Table 1**, and references therein), this dominant negative function of mutant p53 in PDAC may be alternate mechanism to achieve suppression of miR-34b/c. Myc is known to be involved in metastasis[49] and has recently been shown to activate miR-9, a pro-metastatic microRNA,[50] therefore it is possible that miR-34c acts upstream of miR-9 by repressing Myc. In this view miR-34c would lie at the centre of a tumor suppressor network to prevent oncogenic transformation and metastasis by repressing Myc translation (**Fig. 2**).

Future Directions

Several crucial questions remain regarding the regulation of Myc by miR-34c. Does miR-34c inhibit tumor metastasis by repressing Myc? Does genetic loss of miR-34c in vivo lead to tumor formation and is this dependent on Myc? Are there instances in human cancer in which this regulatory mechanism is lost, either through expression of mutant p53 or through a single nucleotide polymorphism (SNP) in the Myc 3' UTR? What function does miR-34c play in the MK2 DNA damage checkpoint? What transcription factor activates miR-34c in the absence of p53? Is therapeutic manipulation of miR-34c feasible? It is possible since therapeutic replacement of miR-34a in a mouse NSCLC xenograft model looks promising[61] and miR-34c is a more potent

anti-proliferative signal than miR34a.[12] Despite these many unanswered questions the available data clearly indicate a central role for miR-34c-dependent regulation of Myc in the prevention of human cancer initiation and progression.

References

1. Cannell IG, Kong YW, Bushell M. How do microRNAs regulate gene expression? Biochem Soc Trans 2008; 36:1224-31.
2. Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. Cell 2009; 136:642-55.
3. Kim HH, Kuwano Y, Srikantan S, Lee EK, Martindale JL, Gorospe M. HuR recruits let-7/RISC to repress c-Myc expression. Genes Dev 2009; 23:1743-8.
4. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. Nature 2008; 455:64-71.
5. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature 2008; 455:58-63.
6. Valastyan S, Benaich N, Chang A, Reinhardt F, Weinberg RA. Concomitant suppression of three target genes can explain the impact of a microRNA on metastasis. Genes Dev 2009; 23:2592-7.
7. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, et al. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 2009; 137:1032-46.
8. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nat Biotechnol 28:341-7.
9. Dorsett Y, McBride KM, Jankovic M, Gazumyan A, Thai TH, Robbiani DF, et al. MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. Immunity 2008; 28:630-8.
10. Teng G, Hakimpour P, Landgraf P, Rice A, Tuschl T, Casellas R, et al. MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. Immunity 2008; 28:621-9.
11. Cannell IG, Kong YW, Johnston SJ, Chen ML, Collins HM, Dobbyn HC, et al. p38 MAPK/MK2-mediated induction of miR-34c following DNA damage prevents Myc-dependent DNA replication. Proc Natl Acad Sci USA 2010; 107:5375-80.
12. He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumor suppressor network. Nature 2007; 447:1130-4.
13. He L, He X, Lowe SW, Hannon GJ. microRNAs join the p53 network—another piece in the tumor-suppression puzzle. Nat Rev Cancer 2007; 7:819-22.
14. Adhikary S, Eilers M. Transcriptional regulation and transformation by Myc proteins. Nat Rev Mol Cell Biol 2005; 6:635-45.
15. Eilers M, Eisenman RN. Myc's broad reach. Genes Dev 2008; 22:2755-66.
16. Cole MD, Cowling VH. Transcription-independent functions of MYC: regulation of translation and DNA replication. Nat Rev Mol Cell Biol 2008; 9:810-5.
17. Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 2008; 40:43-50.
18. Chang TC, Zeitels LR, Hwang HW, Chivukula RR, Wentzel EA, Dews M, et al. Lin-28B transactivation is necessary for Myc-mediated let-7 repression and proliferation. Proc Natl Acad Sci USA 2009; 106:3384-9.
19. O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. c-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005; 435:839-43.
20. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, et al. A microRNA polycistron as a potential human oncogene. Nature 2005; 435:828-33.
21. Dews M, Homayouni A, Yu D, Murphy D, Sevignani C, Wentzel E, et al. Augmentation of tumor angiogenesis by a Myc-activated microRNA cluster. Nat Genet 2006; 38:1060-5.
22. Olive V, Bennett MJ, Walker JC, Ma C, Jiang I, Cordon-Cardo C, et al. miR-19 is a key oncogenic component of mir-17-92. Genes Dev 2009; 23:2839-49.
23. Takakura S, Mitsutake N, Nakashima M, Namba H, Saenko VA, Rogounovitch TI, et al. Oncogenic role of miR-17-92 cluster in anaplastic thyroid cancer cells. Cancer Sci 2008; 99:1147-54.
24. van Haaften G, Agami R. Tumorigenicity of the miR-17-92 cluster distilled. Genes Dev 24:1-4.
25. Dominguez-Sola D, Ying CY, Grandori C, Ruggiero L, Chen B, Li M, et al. Non-transcriptional control of DNA replication by c-Myc. Nature 2007; 448:445-51.
26. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57-70.
27. Reimann M, Loddenkemper C, Rudolph C, Schildhauer I, Teichmann B, Stein H, et al. The Myc-evoked DNA damage response accounts for treatment resistance in primary lymphomas in vivo. Blood 2007; 110:2996-3004.
28. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 1983; 304:596-602.
29. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 2007; 26:745-52.
30. Corney DC, Flesken-Nikitin A, Godwin AK, Wang W, Nikitin AY. MicroRNA-34b and MicroRNA-34c are targets of p53 and cooperate in control of cell proliferation and adhesion-independent growth. Cancer Res 2007; 67:8433-8.
31. Tarasov V, Jung P, Verdoodt B, Lodygin D, Epanchintsev A, Menssen A, et al. Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G_1-arrest. Cell Cycle 2007; 6:1586-93.
32. Herold S, Herkert B, Eilers M. Facilitating replication under stress: an oncogenic function of MYC? Nat Rev Cancer 2009; 9:441-4.
33. Fanidi A, Harrington EA, Evan GI. Cooperative interaction between c-myc and bcl-2 proto-oncogenes. Nature 1992; 359:554-6.
34. Reinhardt HC, Yaffe MB. Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2 and MK2. Curr Opin Cell Biol 2009; 21:245-55.
35. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature 2009; 461:1071-8.
36. Reinhardt HC, Aslanian AS, Lees JA, Yaffe MB. p53-deficient cells rely on ATM- and ATR-mediated checkpoint signaling through the p38MAPK/MK2 pathway for survival after DNA damage. Cancer Cell 2007; 11:175-89.
37. Reinhardt HC, Jiang H, Hemann MT, Yaffe MB. Exploiting synthetic lethal interactions for targeted cancer therapy. Cell Cycle 2009; 8:3112-9.
38. Christoffersen NR, Shalgi R, Frankel LB, Leucci E, Lees M, Klausen M, et al. p53-independent upregulation of miR-34a during oncogene-induced senescence represses MYC. Cell Death Differ 2009; 17:236-45.
39. Kato M, Paranjape T, Ullrich R, Nallur S, Gillespie E, Keane K, et al. The mir-34 microRNA is required for the DNA damage response in vivo in *C. elegans* and in vitro in human breast cancer cells. Oncogene 2009; 28:3008.
40. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 2001; 81:807-69.
41. de Nadal E, Posas F. Multilayered control of gene expression by stress-activated protein kinases. EMBO J 2010; 29:4-13.
42. Prochownik EV. c-Myc: linking transformation and genomic instability. Curr Mol Med 2008; 8:446-58.
43. Ray S, Atkuri KR, Deb-Basu D, Adler AS, Chang HY, Herzenberg LA, et al. MYC can induce DNA breaks in vivo and in vitro independent of reactive oxygen species. Cancer Res 2006; 66:6598-605.
44. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007; 8:729-40.
45. Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, et al. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006; 444:633-7.
46. Morton JP, Timpson P, Karim SA, Ridgway RA, Athineos D, Doyle B, et al. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci USA 2010; 107:246-51.
47. Blagosklonny MV. p53 from complexity to simplicity: mutant p53 stabilization, gain-of-function and dominant-negative effect. FASEB J 2000; 14:1901-7.
48. Muller PA, Caswell PT, Doyle B, Iwanicki MP, Tan EH, Karim S, et al. Mutant p53 drives invasion by promoting integrin recycling. Cell 2009; 139:1327-41.
49. Zhang XY, DeSalle LM, Patel JH, Capobianco AJ, Yu D, Thomas-Tikhonenko A, et al. Metastasis-associated protein 1 (MTA1) is an essential downstream effector of the c-MYC oncoprotein. Proc Natl Acad Sci USA 2005; 102:13968-73.
50. Ma L, Young J, Prabhala H, Pan E, Mestdagh P, Muth D, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010; 12:247-56.
51. Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y, et al. Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res 2008; 68:4123-32.
52. Lee CH, Subramanian S, Beck AH, Espinosa I, Senz J, Zhu SX, et al. MicroRNA profiling of BRCA1/2 mutation-carrying and non-mutation-carrying high-grade serous carcinomas of ovary. PLoS ONE 2009; 4:7314.
53. He C, Xiong J, Xu X, Lu W, Liu L, Xiao D, et al. Functional elucidation of MiR-34 in osteosarcoma cells and primary tumor samples. Biochem Biophys Res Commun 2009; 388:35-40.
54. Lujambio A, Calin GA, Villanueva A, Ropero S, Sanchez-Cespedes M, Blanco D, et al. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA 2008; 105:13556-61.
55. Lodygin D, Tarasov V, Epanchintsev A, Berking C, Knyazeva T, Korner H, et al. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. Cell Cycle 2008; 7:2591-600.
56. Cole KA, Attiyeh EF, Mosse YP, Laquaglia MJ, Diskin SJ, Brodeur GM, et al. A functional screen identifies miR-34a as a candidate neuroblastoma tumor suppressor gene. Mol Cancer Res 2008; 6:735-42.
57. Wei JS, Song YK, Durinck S, Chen QR, Cheuk AT, Tsang P, et al. The MYCN oncogene is a direct target of miR-34a. Oncogene 2008; 27:5204-13.
58. Welch C, Chen Y, Stallings RL. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. Oncogene 2007; 26:5017-22.
59. Corney DC, Hwang CI, Matoso A, Vogt M, Flesken-Nikitin A, Godwin AK, et al. Frequent downregulation of miR-34 family in human ovarian cancers. Clin Cancer Res 2010; 16:1119-28.
60. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 2004; 101:2999-3004.
61. Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. Cancer Res 2010; In press.